EVIDENCE FOR INTERNAL MOBILITY IN ALFALFA MOSAIC VIRUS

P. J. ANDREE, J. H. KAN and J. E. MELLEMA
Department of Biochemistry, State University of Leiden, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands

Received 24 April 1981; revised version received 22 June 1981

1. Introduction

Biological processes are essentially dynamic and, therefore, an understanding of the dynamic aspects of biological structure is essential in obtaining a complete understanding of their function. In the case of small plant viruses the study of structural fluctuations can be of relevance for the mechanisms of the assembly and infection processes.

Our group is engaged in structural studies of alfalfa mosaic virus (AlMV), a small plant virus with a multipartite genome. A preparation of AlMV consists of cylindrical particles of different lengths, roughly proportional with the molecular weight of the RNA enclosed. The isolated coat protein occurs as dimers and its amino acid sequence has been determined [1]. The RNA inside the capsid appears to be loosely packed throughout the particle interior with little penetration into the protein capsid [2].

In this letter ^{1}H-NMR spectra of alfalfa mosaic virus and its coat protein are presented, which indicate that the N terminal part of the peptide chain has extensive internal mobility both in the virus and the isolated protein.

2. Experimental

AlMV was grown, isolated and separated into different molecular weight components as described previously [3,4]. Isolated coat protein was prepared according to Driedonks et al. [5].

NMR spectra were obtained after dialysis against ^2H_2O buffers containing 100 mM deuterated acetate at pH 5.0 and 10 mM sodium phosphate at pH 7.0 for coat protein and intact virus respectively.

Fourier transform ^{1}H-NMR spectra were recorded on a Bruker 360 MHz NMR spectrometer interfaced with a Nicolet computer equipped with a high density disk system. The spectra were obtained at 25°C in a 5 mm NMR tube. In a typical experiment 1000 free induction decays were collected with an acquisition time of 0.8 s and an additional delay of 0.6 s between pulses. Under these conditions the spectra are almost completely relaxed. For quantitative measurements a delay time of 5 s was used. No line broadening or resolution enhancement were applied to the spectra shown.

3. Results and discussion

The ^{1}H-NMR spectrum of the dimeric coat protein is shown in fig.1a. A detailed report of our studies on the coat protein will be published elsewhere. The conclusions that are relevant for the study of the intact virus can be summarized as followed: several resonances in the experimental spectrum at positions marked by asterisks are significantly narrower than is reasonable for a protein of M_r = 48 000. These resonances may be assigned to protons of groups which experience additional motion relative to the whole molecule. A similar situation has been reported for the tobacco mosaic virus coat protein [6] and the *lac* repressor protein [7]. In the two cases cited the motionally narrowed resonances were assigned to a specific region in the amino acid sequence and the same appears to be true in the case presented here: after removing the first 25 residues by mild treatment with trypsin according to Bo et al. [8], a strikingly different spectrum results even though not much more than 10% of the amino acid residues have been removed. In particular, the resonances that can be shown to be motionally narrowed have decreased strongly in intensity after trypsin treatment and are, therefore, assigned to the N-terminal part which

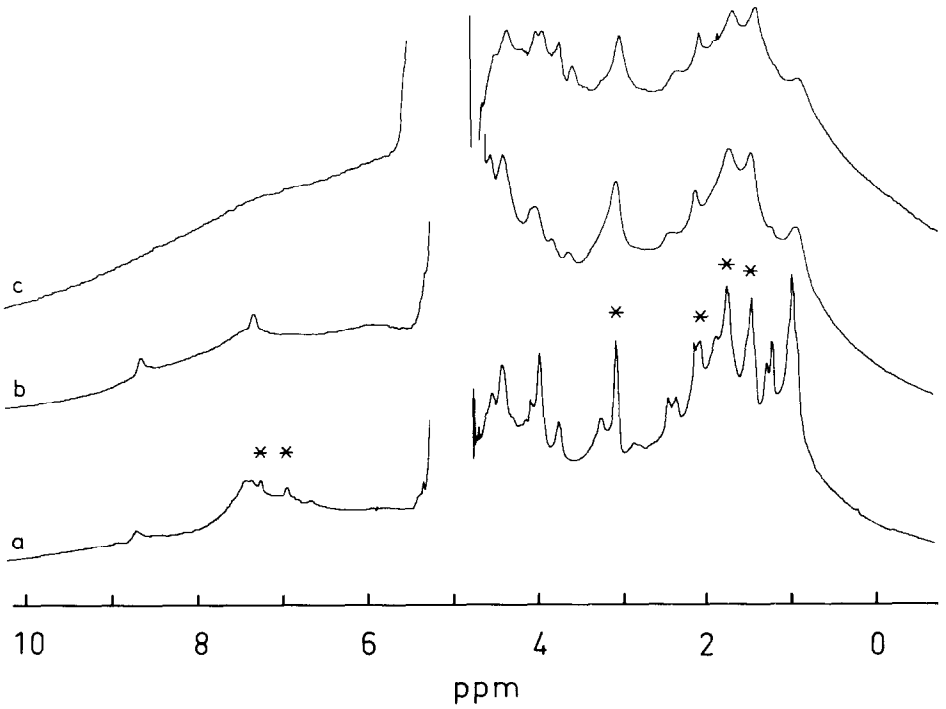

Fig.1. 360 MHz NMR spectra of (a) isolated dimeric coat protein of AlMV 425; (b) top component of AlMV 425; (c) long particles of strain VRU. The spectra were obtained in ^2H_2O buffers containing: (a) 100 mM deuterated sodium acetate at pH 5.0; and (b), (c) 10 mM sodium phosphate at pH 7.0 (meter reading).

appears to be flexible. In fact, many of the motionally narrowed resonances could be specifically assigned to residues in the N-terminal part on the basis of spectral simulations. The highest intensity peaks in the aliphatic region mainly stem from groups of lysine and arginine residues, which are particularly abundant in this part of the sequence. The aromatic resonances at 7.0 and 7.3 ppm are from Tyr 21.

The dynamic properties of the coat protein seem to extend to the intact virus. In fig.1b and c, spectra are shown for the strain AlMV 425 top (smallest) component (P. W. = 3.54×10^6 daltons [9]) and the strain VRU from which a molecular mass fraction, containing long rod-like particles, has been isolated with a particle weight at least 10 times higher than that of 425 top component. Resolved resonances in the aliphatic region of the virus spectrum are observed at positions corresponding with the motionally narrowed resonances in the protein spectrum. The discrepancy of the measured line width and the theoretical expectation [10] for a rigid virus particle is in the order of a factor 100. Therefore it appears that the N-terminal part of the coat protein in assembled virus is in a state of dynamical disorder. It can be assumed, on the basis of its basic character that the N-terminal part plays a role in the interaction with RNA as has been found for several other systems. However, there are no sharp lines in the aromatic region that could suggest that the viral RNA has a comparable mobility. It cannot be excluded that the resolved lines stem from a fraction of the protein molecules. Non-equivalence of the protein units in an assembled virus particle is likely on the basis of symmetry arguments, as first pointed out by Caspar and Klug [11]. AlMV coat protein in the cylindrical lattice (plane group P6) and protein organized in the T = 1 icosahedral lattice at the rounded caps have, necessarily, different environments. Experimental evidence for a comparable case of non strict equivalence in packing of protein in large (T # 1) spherical shells has been found by X-ray diffraction studies [12,13]. It is, therefore, not unlikely that the motionally narrowed lines from the N-terminal part of the protein in assembled virus arise from a fraction of coat protein molecules possibly not

engaged in protein–RNA interaction, but the fact that resonances from Tyr 21 are clearly observed in the coat protein spectrum but not in the intact virus argue against this possibility.

The well-resolved lines in the spectrum appear to be superimposed on a broader component which is still narrower than would be expected in the case of complete rigidity. Integration of the spectrum and comparison with an internal DSS standard shows that the total area corresponds with the majority of the protons in the virus. The possibility that we observe only a small portion of possibly denatured material is therefore excluded. The similarity of the spectra of virus particles from different strains which differ by an order of magnitude in molecular mass is another indication that the NMR relaxation parameters are not determined by the motion of the particle as a whole, and that the spectral shape is determined by the presence of internal mobility in the virus. This result is in agreement with those obtained by de Wit et al. [10], who observed internal mobility in AlMV and several other plant viruses and ribosomes. However, other reports are indicative of more rigid virus structures. Jardetzky et al. [6], reported an unusual flexibility in a region of the coat protein of tobacco mosaic virus and indicated that this mobility is required to enable the assembly with RNA whereafter this segment is immobilized. Recently, Munowitz et al. [14] reported, on the basis of ^{31}P-NMR spectra, that the RNA in tomato bushy stunt virus was rigid in the native state but that dynamic disorder was present after EDTA treatment which causes a swelling of the virus. In this respect it might be of relevance that no swelling could be induced in AlMV while this virus in several of its properties resembles the swollen forms of viruses like brome mosaic virus, cowpea chlorotic mottle virus and southern bean mosaic virus rather than the compact form [15].

Two resolved resonances are observed in the aromatic region of the AlMV 425 spectrum which are absent in the spectrum of the VRU strain. The lines occur at 7.4 and 8.8 ppm, which are typical values for the C-4 and C-2 protons of a protonated histidine residue. Comparison of the amino acid sequence of the coat proteins of the two different strains [16] shows that the only histidine present in the former and absent in the latter strain is the C-terminal residue His-220. Integration of these peaks was rather inaccurate but the results are at least consistent with assignment to single protons. We assign these two resonances, therefore, to His-220, which is the first time that such a specific assignment could be made in the ^{1}H-NMR spectrum of an intact virus.

The spectrum of the coat protein of strain VRU has essentially the same features as the spectrum shown in fig.1a. Therefore, in the case of isolated coat protein there is no indication that one specific histidine residue has additional mobility. The fact that such mobility is observed at the C-terminus of intact virus and not for the isolated coat protein could be taken as suggestive evidence for a conformational change of the protein when it is assembled in the intact virus.

4. Conclusion

The usefulness of NMR techniques in investigating the dynamic properties of large molecular assemblies like a virus is demonstrated in this study which shows that the N-terminal part of a large part or all of the coat protein molecules in assembled AlMV are in a state of dynamical disorder. The possible functional significance of this dynamic property for the assembly and infection processes are subject of further investigations.

Acknowledgements

The authors thank B. M. de Koster for his skillful technical assistance with the virus isolation. The work was supported by a grant from The Netherlands Foundation for Chemical Research (SON). Use was made of the SON supported high resolution NMR facility at Groningen. The assistance of Dr R. Kaptein and K. Dijkstra with the NMR measurements is gratefully acknowledged.

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